The impact of the hydroxyl radical photochemical sources on the rivastigmine drug transformation inmimic and natural waters

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Abstract

In this paper we investigated the degradation of the rivastigmine drug induced by hydroxyl radical in synthetic and natural waters focusing on both reactivity and photoproduct identification. The hydroxyl radical formation rate was quantified by using terephthalic acid as trapping molecule and it was related with the rivastigmine degradation rate. The second order rate constant between hydroxyl radical and rivastigmine was estimated to be ~ 5.8×10^9 M^{-1} s^{-1}. Irradiation of rivastigmine in three natural waters (rain, lake and river) and comparison with degradation rates observed in synthetic solutions using nitrite, nitrate and hydrogen peroxide suggest that, in addition to hydroxyl radical, also nitroderived radicals ($NO^{\bullet}/NO_2$) are responsible for the pollutant degradation in natural media. In fact, the evaluated degradation rates in three natural waters are greatly higher than those estimated considering only the reactivity with photogenerated hydroxyl radical. Using nitrites and nitrates as photochemical $^{\bullet}OH$ source, the rivastigmine degradation cannot be described considering

only the hydroxyl radical reactivity suggesting that NO and NO_2 radicals could play a key role during indirect degradation. Moreover main degradation products have been identified by means of HPLC-MS. Hydroxylation of the aromatic ring as well as carbamate and amino chain oxidation were suggested as main reaction mechanisms, but also nitroderived compounds were characterized.

Finally polychromatic irradiations of three rivastigmine doped natural waters (rain, river and lake) underlined the role of the indirect degradation that needs to be considered when direct degradation of selected pollutants is negligible under environmental-like conditions.

Keywords: Photoinduced degradation; environmental fate; radical-induced transformation; photoxidation; hydroxyl radical

1. Introduction

In recent years, the environmental pollution by emerging pollutants has become a major issue and it is now well established that human pharmaceuticals effects might potentially play a major role in other (non-mammalian) eco-organisms once reached the natural media (deep and surface waters, soils ,…) (Khetan and Collins 2007). On the other hand, the impact and fate of xenobiotics need to be investigated in order to asses the environmental potential risk. Furthermore, the investigation of the environmental fate of different kinds of pharmaceuticals is one of the main goals of recent European projects (Pharmas and Cytothreat). As stated in the summary of the Pharmas project a classification system needs to be developed with the intention of enabling all EU citizens to make their own informed decisions about the risk posed by human pharmaceuticals to their health and to the health of the environment. The results of this investigation will able EU regulators and policy makers to make better

informed decisions on the issue of pharmaceuticals in the environment (RIFERIMENTO_ link? http://www.pharmas-eu.org/).

In addition to priority pollutants many organic compounds used for human and veterinary health, known as pharmaceuticals and personal care products (PPCPs), are regularly introduced into the environment (McClellan and Halden 2010, Richardson and Ternes 2005, Yu and Chu 2009). Many of these compounds, after application, are excreted by urine and feces and are transported into sewage (Farré et al. 2008, Kümmerer et al. 1997). The sewage treatment plants (STPs) are often unable to completely remove PPCPs that in this way enter the environment, where they are frequently found (Andreozzi et al. 2003, Ellis 2006, Murray et al. 2010). In the environment the pollutants may undergo biotic and/or abiotic transformations (Cermola et al. 2007, Della Greca et al. 2008, DellaGreca et al. 2006, Jjemba 2006). In the case of surface waters, photochemical processes are the most important among abiotic transformations. In such medium the indirect photolysis involving oxidant species (singlet oxygen, hydroxyl radical, photoexcited organic matter) could be relevant for the complete transformation of such compounds. The transformation products may be more persistent and exhibit higher toxicity than parent compounds (DellaGreca et al. 2004). Drugs are compounds made to have a biological activity and in the environment, even at small concentration, they can interact with aquatic or non aquatic organisms with effects, often unknown, on the ecosystem (Daughton and Ternes 1999, Halling-Sørensen et al. 1998, Jørgensen and Halling-Sørensen 2000, Vaal et al. 1997). PPCPs include many classes of heterogeneous compounds. More than 3000 pharmaceutical ingredients are registered (Richardson et al. 2005, Ternes et al. 2004) and it is hard to predict the environmental fate and the critical concentration for most of these compounds.

Many studies in the literature are about direct photodegradation of pollutants in organic solvents and in water, identification of their photoproducts and the related formation

77 mechanisms (Burrows et al. 2002, de Bertrand and Barceló 1991, Doll and Frimmel 2003,
78 Sanz-Asensio et al. 1999). In order to predict the environmental fate of a pollutant it is
79 essential to study indirect photolysis as well, which is often the most important process. In
80 fact, in natural waters the pollutants may react with oxidant species more rapidly than with
81 direct light. Only few studies determine the values of second order kinetic constants with $^{\bullet}OH$
82 (Charbouillot et al. 2012, Razavi et al. 2011), one of the most important oxidants in natural
83 waters.
84 Rivastigmine (Riv) is a drug used to treat patients with Alzheimer's disease and dementia
85 associated with Parkinson's disease (Mustazza et al. 2002). Rivastigmine hydrogen tartrate is
86 one of the few drugs approved in 2000 to treat Alzheimer's disease. This drug is a
87 cholinesterase inhibitor and acts on the central nervous system. One active site of the
88 molecule is the carbamate function (Mustazza et al. 2002, Yao and Li 2008), a function often
89 present in drugs and pesticides (insecticides and herbicides) (Gupta and Ramesh 2006).
90 Compounds with a carbamate function may undergo hydrolysis in the environment, but many
91 of these are found in ground and surface waters (Campbell et al. 2004, García de Llasera and
92 Bernal-González 2001).
93 Recently we investigated the direct degradation of rivastigmine under mono- and
94 polychromatic wavelengths underlining the phototransformation under environmental-like
95 conditions (Temussi et al. 2012).
96
In this work the transformation of rivastigmine toward hydroxyl radical generated using
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different natural sources (H_2O_2, NO_3^-, NO_2^-) was investigated showing the possible enhanced
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transformation attributed to the $NO_2^{\bullet}$ and $NO^{\bullet}$ reactivity. Moreover three irradiations were
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performed in natural waters (lake, river and rain) in order to compare the rivastigmine
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degradation with previously obtained results in synthetic waters. A particular attention was

given to the degradation products showing the synergic effect of hydroxyl and NOx radicals on the rivastigmine transformation in waters.

2. Materials and Methods

2.1 Chemicals

Rivastigmine hydrogen tartrate (99 %) was purchased by Kemprotec. Hydrogen peroxide (30 %), sodium carbonate (99.5 %) and sodium nitrate (99 %) were purchased from Fluka; sodium sulphate anhydrous (99.5 %) and sodium nitrite (98%) were purchased from Prolabo; phosphoric acid (85% in water), sodium chloride (98 %), terephthalic acid (98 %) and 2-hydroxyterephthalic acid (97 %) were purchased from Sigma Aldrich without additional purification. All solvents were of HPLC grade and were purchased from Sigma-Aldrich.

Fresh solutions doped with nitrate, nitrite or H_2O_2 were prepared before each experiment. The concentration of the stock solution of H_2O_2 in milli-Q water was determined using a molar absorption coefficient of $38.1 \pm 1.4\ M^{-1}\,cm^{-1}$ at 240 nm (Miller and Kester 2002).

2.2 Irradiation experiments

Aqueous solutions were irradiated in a thermostated cylindrical reactor. The 40 mL reactor, cooled by water circulation at a temperature of 15 ± 2 °C in order to limit thermal reactions, was located at one focal point of the lamp in order to maintain a constant irradiation of the whole sample and it was equipped on the top with a pyrex filter removing the wavelengths lower than ~285 nm. Samples were continuously stirred with a magnetic stirrer and a Teflon bar to ensure homogeneity.

The emission spectrum of the Xenon lamp (see Figure 1) was recorded using a fiber optics coupled with a CCD spectrophotometer (Ocean Optics USD 2000+UV-VIS). A reference lamp (DH-2000-CAL, Ocean Optics) was used for calibration. The emission spectrum

reaching the reactor surface was calculated to be 4.31×10^{19} photons cm^{-2} s^{-1} over the wavelength range 290-400 nm.

2.3 Detection of hydroxyl radicals

The hydroxyl radical formation rate ($R^f_{\bullet OH}$) was determined by using terephthalic acid (TA) as trapping molecule. TA reacts with hydroxyl radical leading to the formation of 2-hydroxyterephthalic acid (TAOH) with a yield (Y_{TAOH}) determined as the ratio between the initial formation rate of TAOH (R^f_{TAOH}) and the initial degradation rate of TA (R^d_{TA}). Y_{TAOH} ranged between 12 and 30% depending on the pH and temperature of aqueous media (Charbouillot et al. 2011). This method represents a simple and fast detection of photogenerated •OH with a high sensitivity that allows detection of less the 10^{-18} M of hydroxyl radical steady state concentration.

In order to calculate the formation rate of •OH as a function of different aqueous media composition, different solutions were irradiated with the same setup used for rivastigmine photochemical experiments. An aliquot of solution (3 mL) was withdrawn and put in a fluorescence cuvette at fixed times. The cuvette was transferred into a Perkin-Elmer MPF 3 L spectrofluorimeter and TAOH was quantified by using a calibration curve previously made with standard solutions of TAOH. The concentration of TA used during all experiments was included between 500 µM and 1 mM in order to trap all photogenerated •OH.

2.4 Natural waters

Lake, river and rain waters, sampled on 2011, were collected in order to perform hydroxyl radical measurement and rivastigmine degradation studies. River water and lake water come from Artiere river and Chambon lake, respectively, both located in the Puy de Dôme region, France. Artiere river passes through Clermont-Ferrand agglomeration, Chambon lake is in the

151 massif of Dore mountains at 877 m above sea level. Rain water was collected on the 1st
152 November 2011 at the "Cézeaux campus" (south of Clermont-Ferrand) located at 394 m of
153 altitude on the south of Clermont-Ferrand city.
154 Natural waters samples, collected using a glass bottle, were filtered on 0.20 µm membranes
155 (Minisart®, Sartorium Stedim) and stored under refrigeration. Irradiation experiments were
156 performed the day after, while, for analysis by ion chromatography (IC) inorganic and organic
157 anions concentrations were determined no more than 24 hours after sampling.
158
159 **2.5 Photoinduced degradation and data analysis.**
160 The experiments were performed either in Milli-Q water or in natural waters. The solutions in
161 Milli-Q water were prepared by mixing different concentrations of hydrogen peroxide (188
162 µM, 376 µM, 550 µM 752 µM), or nitrate (200 µM, 400 µM, 5 mM) or nitrite (5.4 µM, 10
163 µM, 50 µM, 100 µM) to a constant concentration of rivastigmine (45 µM). In order to analyze
164 the time evolution of rivastigmine an aliquot (200 µL) was taken each time, stored in the dark
165 and analysed by HPLC-UV. An HPLC system (Waters Alliance) equipped with a diode array
166 detector was used for rivastigmine analysis. An Eclipse XDB-C18 column (Agilent, 4.6 x 150
167 mm, 5 µm) and a gradient elution at a flow rate of 1.0 mL min^{-1} were used. The gradient was:
168 at initial time 10 % acetonitrile and 90 % water acidified with 3‰ phosphoric acid, it was
169 kept for 7 min, and then followed by a linear gradient to 90 % acetonitrile within 8 min. Then,
170 the same ratio was maintained constant for 8 min followed by a linear gradient to 10 %
171 acetonitrile within 2 min. This ratio was maintained constant for 5 min. UV detector was set
172 at 210 nm. To prevent reactions after sampling, amber glass vials were used. Retention time
173 of rivastigmine was 11.2 min.
174 The time evolution of rivastigmine in the presence of photochemical sources of •OH could be
175 fitted with a pseudo-first order equation $C_0 = C_t \exp(-k\,t)$ where C_0 was the initial

176 rivastigmine concentration, C_t the concentration at time *t* and k the pseudo-first order
177 degradation rate constant. The error bars associated to the rate data represent 3σ, derived from
178 the scattering of the experimental data around the fitting curves (intra-series variability).
179 Ion chromatography analyses were performed using a DIONEX DX-320 instrument equipped
180 with an IonPac AG11 (guard-column 4 × 50 mm) and an IonPac AS11 (analytical column 5 ×
181 250 mm) for anions. The elution step was performed using the following KOH concentration
182 gradient: at initial time [KOH] = 0.2 mM increasing up to 0.43 mM at 4.5 min, followed by a
183 linear gradient to 11.7 mM up to 18 min. The [KOH] was then linearly increased up to 33.5
184 mM within 3 min. The flow rate was 1 mL min^{-1}.
185 Pure rivastigmine solution was obtained by dissolving the tartrate salt in a saturated Na_2CO_3
186 aqueous solution. The solution was then extracted using ethyl acetate (3 x 10 mL) and the
187 combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4, before
188 filtration and evaporation under vacuum. Absence of tartrate was confirmed by NMR
189 spectroscopy and pure rivastigmine was stored in the dark at 5°C. Riv stability was checked
190 by comparison with UV-vis spectrum (recorded from 250 to 450 nm) of fresh extracted
191 solution (see Figure 1). pH values were measured with a Metrohm combined glass electrode,
192 connected to a Metrohm 713 pH-meter.
193 Hydrogen peroxide concentration was determined by spectrophotometric absorbance
194 detection of a solution in which *N*,*N*-diethyl-*p*-phenylenediamine (DPD) was involved in a
195 reaction catalysed by a peroxidase (POD). In the presence of POD, H_2O_2 oxidizes two
196 molecules of DPD to cationic radicals $DPD^{\bullet+}$ (Bader et al. 1988), which are stable and absorb
197 light at 551 nm (ε = 21 000 M^{-1} cm^{-1}). With a 10 cm optical path length cell, the detection
198 limit was 0.1 µM calculated as 3 times the standard deviation of field blanks. Each
199 measurement took less than 5 minutes.

Total organic carbon (TOC) and HCO_3^-/CO_3^{2-} concentrations in natural waters were quantified by TOC-5050A Analyzer (Shimadzu) using calibration curves previously performed on standard solutions.

2.6 Phototransformation products identification

Degradation products identification was performed by means of an LC-MS system (Agilent 1100 Series, binary pump) equipped with an ESI ion source (MSD VL). The adopted column was a Sphere Clone C18 column (Phenomenex, 4.6 x 250 mm, 5 µm) and the following gradient elution was used: at initial time 30 % acetonitrile and 70 % water acidified with 1% formic acid, followed by a linear gradient to 75 % acetonitrile within 55 min. Then, the same ratio was maintained constant for 20 min. The flow rate was 0.4 mL min^{-1} and the UV detector was settled at 254 nm.

3. Results and discussion

The goal of this work is o was? to assess the reactivity of rivastigmine toward a wide range of photochemical precursors of hydroxyl radical in the aquatic environment upon irradiation of laboratory-made and natural solutions.

3.1 Second order rate constant of rivastigmine with hydroxyl radical

The second-order rate constant between Riv and hydroxyl radical was calculated by competition kinetics with isopropanol ($(CH_3)_2CHOH$, 2Pr) added to the solution at different concentrations. In Figure 2 we report the degradation rate of Riv obtained by irradiation of a solution containing 45 µM of Riv, 380 µM of H_2O_2 and with addition of different isopropanol concentrations ranging from 0 up to 1 mM. H_2O_2 was used as source of hydroxyl radical via its photolysis (R1) under polychromatic irradiation.

The following reactions were included in the kinetic treatment of data reported in this work:

$$H_2O_2 \xrightarrow{h\nu} 2\ {}^{\bullet}OH \qquad [R^{f}_{{}^{\bullet}OH}] \qquad \text{(R1)}$$

$$Riv + {}^{\bullet}OH \rightarrow \text{Products} \qquad [k_{Riv,{}^{\bullet}OH}] \qquad \text{(R2)}$$

$$(CH_3)_2CHOH + {}^{\bullet}OH \rightarrow (CH_3)_2\dot{C}OH + H_2O \qquad [k_{2Pr,{}^{\bullet}OH} = 1.9 \times 10^9\ M^{-1}\ s^{-1}] \qquad \text{(R3)}$$

$$H_2O_2 + {}^{\bullet}OH \rightarrow H_2O + HO_2^{\bullet} \qquad [k_{H_2O_2,{}^{\bullet}OH} = 2.7\times 10^7\ M^{-1}\ s^{-1}] \qquad \text{(R4)}$$

The experimental data were fitted by using the following equation (eq1).

$$R^{d}_{Riv} = \frac{R^{f}_{{}^{\bullet}OH}\ k_{Riv,{}^{\bullet}OH}[Riv]}{k_{Riv,{}^{\bullet}OH}[Riv] + k_{H_2O_2,{}^{\bullet}OH}[H_2O_2] + k_{2Pr,{}^{\bullet}OH}[2Pr]} \qquad \text{(eq 1)}$$

The data reported in Figure 2 were fitted with an equation of the type: $y = \frac{a}{1+bx}$ where a is the ratio $\frac{R^{f}_{{}^{\bullet}OH}\ k_{Riv,{}^{\bullet}OH}[Riv]}{k_{Riv,{}^{\bullet}OH}[Riv] + k_{H_2O_2,{}^{\bullet}OH}[H_2O_2]}$, b is $\frac{k_{2Pr,{}^{\bullet}OH}}{k_{Riv,{}^{\bullet}OH}[Riv] + k_{H_2O_2,{}^{\bullet}OH}[H_2O_2]}$ and x the concentration of isopropanol. From the data fit we can obtain the second-order reaction rate constant between hydroxyl radical and rivastigmine: $k_{Riv,{}^{\bullet}OH} = \frac{\frac{k_{2Pr,{}^{\bullet}OH}}{b} - k_{H_2O_2,{}^{\bullet}OH}[H_2O_2]}{[Riv]}$

To the best of our knowledge, $k_{Riv,{}^{\bullet}OH}$ was determined for the first time to be (5.8 ± 0.3) × 10^9 M^{-1} s^{-1}. Additionally we obtained the formation rate of ${}^{\bullet}OH$ from H_2O_2 photolysis under the adopted irradiation condition: $R^{f}_{{}^{\bullet}OH} = \frac{a\,(k_{Riv,{}^{\bullet}OH}[Riv] + k_{H_2O_2,{}^{\bullet}OH}[H_2O_2])}{k_{Riv,{}^{\bullet}OH}[Riv]} = 9.6 \times 10^{-11}$ M s^{-1} in agreement with results obtained via direct quantification using TA.

3.2 Reactivity toward different hydroxyl radical sources

The stability of rivastigmine in aqueous solution was checked under dark conditions at pH 6.0 and no significant degradation was observed after 1 month, moreover direct photogredation results to be negligible under our irradiation conditions. Rivastigmine (45 μM) was then irradiated in the presence of different amounts of hydrogen peroxide, nitrate and nitrite. First of all the hydroxyl radical formation rate (R^f_{OH}) was estimated as function of the photochemical concentration by using 1 mM TA as reported in Table 1.

As expected, the degradation rate of rivastigmine (R^d_{Riv}) increases with the increasing of the concentration of the photochemical source of $^{\bullet}OH$. In Figure 3 we report the initial degradation rate of rivastigmine R^d_{Riv} as function of hydrogen peroxide and nitrite concentration. In the case of hydrogen peroxide a competition due to the reaction R4 could be predictable. Moreover using nitrite as $^{\bullet}OH$ precursor the source reacts with photogenerated hydroxyl radicals leading to the formation of nitrogen dioxide ($NO_2^{\bullet}$) with a second order rate constant $k_{NO2-,\bullet OH} = 1.0 \times 10^{10}\ M^{-1}\ s^{-1}$ (Mack and Bolton 1999). The plateau observed in figure 3 using 50 μM of nitrite could be explained taking into account its competitive role at high concentrations toward photogenerated hydroxyl radical. In fact, considering the initial concentration of Riv (45 μM) and nitrite (50 μM) in water we can argue that 34 % of hydroxyl radical reacts with Riv while up to 66 % with nitrite. Additionally the percentage significantly changes using 10 μM of nitrite. Under such conditions the 73 % of $^{\bullet}OH$ reacts with Riv while only 28 % with nitrite. Using nitrite and/or hydrogen peroxide a fraction of photogenerated hydroxyl radical reacts also with their sources. From this assumption it is then possible to quantify the fraction of hydroxyl radical reacting with rivastigmine for each experimental point considering the initial concentrations of all species present in the solution and related second order rate constants with $^{\bullet}OH$. Therefore the correlation of the hydroxyl radical formation rate (generated form hydrogen peroxide, nitrite and nitrate photolysis)

reacting with Riv??? is reported in Figure 4 showing that, under adopted concentrations, the dependences are linear as expected. Nevertheless, using nitrite and nitrate, the rivastigmine degradation increases compared to the value obtained in the presence of hydrogen peroxide. Such trend could be explained considering the additional reactivity of $NO_2^{\bullet}$ (and probably $NO^{\bullet}$) generated via nitrate and nitrite direct photolysis or reaction with hydroxyl radical leading to the formation of both hydroxylated and nitro-derived compounds (Vione et al. 2001, 2004). Such radicals could directly react with Riv and/or with radical intermediates generated after H-abstraction driven by photogenerated hydroxyl radical. In any case nitroderived radicals are generated and their reactivity toward Riv can explain the degradation enhancement discussed in the following section.

3.3 Product identification

Three experiments were conducted using a rivastigmine concentration of 4 mM in the presence of hydrogen peroxide 3.5 mM (experiment A), nitrates 15 mM (experiment B) and nitrite 50 µM (experiment C). After 48 hours of irradiation the solutions were analyzed using an HPLC-UV-MS.

Three main reaction-type mechanisms were discerned and the structures of the nineteen identified products are proposed in Figure 5 on the basis of their mass spectra. The main reaction, as expected in the presence $^{\bullet}$OH precursors, is the hydroxyl radical addition to the aromatic ring leading to the formation of product **A1**, while di- and tri- hydroxylated compounds (**A2** and **A3**) are successively generated. Alcohol (**A4**), ketone (**A5**) and vinyl (**A6**) derivatives could also be formed from direct photolysis via a homolytic and/or heterolytic cleavage of the N-C bond as recently reported (Temussi et al. 2012) or via hydroxyl radical attach as shown during mineralization of sulfamethoxazole (Wang et al. 2011).

Hydroxylation of **A4** and **A5** leads to the formation of **A7** and **A8** found only in trace levels under the adopted irradiation conditions.

In the presence of nitrite and nitrate two species with m/z $[M+H]^+$ = 280 and 310 suggest that nitration of the aromatic ring (**B1** and **B2**) could occur (Nélieu et al. 2004).

Few products were also found under all experimental conditions, with m/z $[M+H]^+$ = 121, 123, 137, 166, 178 and 180 obtained from the carbamate chain cleavage or hydroxylation of previously generated oxidation products. Moreover, high mass values were found (m/z = 411, 429, 445 and 467) suggesting the formation of dimer molecules produced *via* radical recombination.

3.4 Irradiation in Natural waters

Three natural waters (rain, lake and river) were doped with Riv (45 µM) in order to investigate the role of hydroxyl radical sources on the rivastigmine degradation.

Inorganic anions, TOC and carbonates concentrations are reported in Table 2. The main differences are the nitrate and nitrite concentrations found in river water: 231 µM and ~7 µM respectively, while in rain and lake they are present only at µM levels or under the detection limit. Also carbonates are more concentrated in river as well as chlorine and sulphate ions, while similar TOC concentrations are found in the three samples.

Hydroxyl radical formation rate (R^f_{OH}) measured in lake and rain water using TA as trapping probe (Charbouillot et al. 2011) are in agreement with previously reported values in literature (Albinet et al. 2010, Vione et al. 2010). The calculated formation rate values range from 2.0 × 10^{-11} up to 6.5 × 10^{-11} M s^{-1} and from 3.2× 10^{-12} to 4.0 × 10^{-11} M s^{-1} for rain and lake waters, respectively. In our experiment the R^f_{OH} in river water was 6-7 times higher than in lake and rain. Such value could be explained considering the high nitrate and nitrite concentration in river samples. Moreover measured R^f_{OH} is well estimated showing that nitrite and nitrate

316 contributions represent about 57 and 22 % of the total hydroxyl radical formation in such
317 medium. Nevertheless for rain and lake waters about 47 and 98 % of hydroxyl radicals are
318 produced by other photochemical sources such as free iron, iron-complexes or chromophoric
319 dissolved organic matter (CDOM) via its excited state (CDOM*). The possible impact of the
320 carbonate radicals on the rivastigmine degradation rate founded in river water could be
321 neglected. In fact carbonates can act as hydroxyl radical scavengers, generating carbonate
322 radical, which could induce organic molecule degradation via electron transfer reaction and/or
323 hydrogen abstraction with a quite low second order rate constant (10^{-6}-10^{-7} M^{-1} s^{-1}) compared
324 to $^{\bullet}OH$. Concerning the "scavenging role", if we consider the relative low carbonates
325 concentrations founded in natural waters used in this work (in river water we have 1 mM
326 while for others the concentration not exceed 1.5×10^{-4} M) we can argue, taking into account
327 the carbonate (HCO_3^-/CO_3^{2-}), nitrite and Riv initial concentrations and the second order rates
328 constants with $^{\bullet}OH$ ($k_{HCO3--,\bullet OH}$ = 8.5 x 10^6 M^{-1} s^{-1} (Buxton et al. 1998) $k_{NO2-,\bullet OH}$ = 1.0 x 10^{10}
329 M^{-1} s^{-1} (Mack and Bolton 1999) and $k_{Riv,\bullet OH}$ = 5.8 × 10^9 M^{-1} s^{-1} (this work)) that, in river
330 water, about 0.25% of photogenerated hydroxyl radicals reacts with hydrogenocarbonate (at
331 pH 7.9 more then 98% of total carbonate species are hydrogenocarbonate), ~22 % with nitrite
332 and up then 78 % with rivastigmine.
333
334 **3.5 Radical driven degradation in natural selected waters**
335 In order to estimate the fate of the selected pollutant in natural waters, considering the double
336 role of naturally occurring compounds, the hydroxyl radical steady-state concentration
337 $[^{\bullet}OH]_{ss}$ was measured in three natural waters by using TA (1 mM) as chemical probe
338 following equation 2:
339 $$-\frac{d[TA]}{dt} = k_{TA,\bullet OH}[TA][^{\bullet}OH]_{ss} \qquad \text{(eq 2)}$$

where [TA] and -d[TA]/dt are the initial concentration and the degradation rate of TA respectively, $k_{TA,^{\bullet}OH}$ the second order rate constant between the probe and photogenerated hydroxyl radical (3.9×10^{9} M^{-1} s^{-1}) (Charbouillot et al. 2011). The $[^{\bullet}OH]_{ss}$ values range from 1.4×10^{-18} M for Lake up to 1.5×10^{-17} M for River water (Table 3). Experimental results are in agreement with those reported by Brezonik and Fulkerson-Brekken (Brezonik and Fulkerson-Brekken 1998) in 5 natural waters (river and lakes) for June mid-day sunlight radiation. The authors reported $[^{\bullet}OH]_{ss}$ ranging from 2.5×10^{-17} up to 1.0×10^{-15} M and 9.0×10^{-19} to 5.5×10^{-17} M in surface and whole water column respectively.

The hydroxyl radical steady-state concentration allows us the estimation of the Riv degradation rate considering only the hydroxyl radical reactivity and scavenging effect of naturally present compounds such as nitrite, carbonates (CO_3^{2-}/HCO_3^{-}) and CDOM using equation 3.

$$R_{Riv}^{d,^{\bullet}OH} = k_{Riv,^{\bullet}OH}[Riv][^{\bullet}OH]_{ss} - \sum_i k_{i,^{\bullet}OH}[S_i][^{\bullet}OH]_{ss} \qquad \text{(eq 3)}$$

Where $R^{d,}{}_{Riv}{}^{OH}$ and [Riv] are the degradation rate of rivastigmine due to the $^{\bullet}OH$ and its concentration, $\sum_i k_{i,^{\bullet}OH}[S_i]$ is calculated as the sum of the contribution of the initial concentration of scavengers (nitrite, carbonates and CDOM) with the reaction rate constant with hydroxyl radical reported above. For these calculations the second order rate constant between CDOM and hydroxyl radical of 2.3×10^{4} mg of C L s^{-1} (?ignoranza mia . che significa?) (Brezonik and Fulkerson-Brekken 1998) has been used. The R^{d}_{Riv} (M s^{-1}) estimated considering only the role of hydroxyl radical using equation 3 (eq. 3) results to be less than those measured during irradiation in natural waters showing that additional degradation pathways are responsible for the rivastigmine degradation as observed under irradiation in synthetic waters.

Using the second order rate constant between hydroxyl radical and rivastigmine (5.8×10^9 M^{-1} s^{-1}) founded in this work and highest $^{\bullet}OH$ steady-state concentrations calculated in some natural waters (10^{-15}-10^{-16} M) (Brezonik and Fulkerson-Brekken 1998, Vione et al. 2010) the $t_{1/2}$ of rivastigmine using equation 4

$$t_{1/2} = \frac{\ln 2}{k_{Riv,^{\bullet}OH}[^{\bullet}OH]_{ss}} \qquad \text{(eq 4)}$$

could be estimated between 1 day up to 14 years?????. Nevertheless, as reported in the text the real degradation is expected to be considerably enhanced due to the presence of nitroderived reactive species.

4. Conclusions

The degradation of rivastigmine drug was investigated toward different photochemical radical sources found in natural waters. The first correlation of the formation rate of hydroxyl radical underlines a participation of radicals such as $NO_x^{\bullet}$ during polychromatic degradation in mimic and natural media. The chemical structures of the main products generated *via* oxidation of Riv have been suggested and hydroxylation, dimethylamino and carbamate chains degradation as well as nitration have been identified as possible pathways to explain the degradation products formation. Moreover the irradiation of Riv in three natural waters underlines a key role of indirect degradation in natural media when direct photolysis is negligible. The discrepancies between the Riv degradation rates calculated considering only the effect of hydroxyl radical and those measured in three natural waters underline the possible enhancement of the pollutant degradation in the presence of naturally occurring compounds such as nitrate and nitrite. Such reactivity could sensibly accelerate the pollutant degradation and need to be considered for a realistic half-life time estimation of organic pollutants in natural media.

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Figure Caption

1) Emission spectrum reaching the solution and molar absorption coefficients of the aqueous rivastigmine, nitrate, nitrite and H_2O_2 solutions.

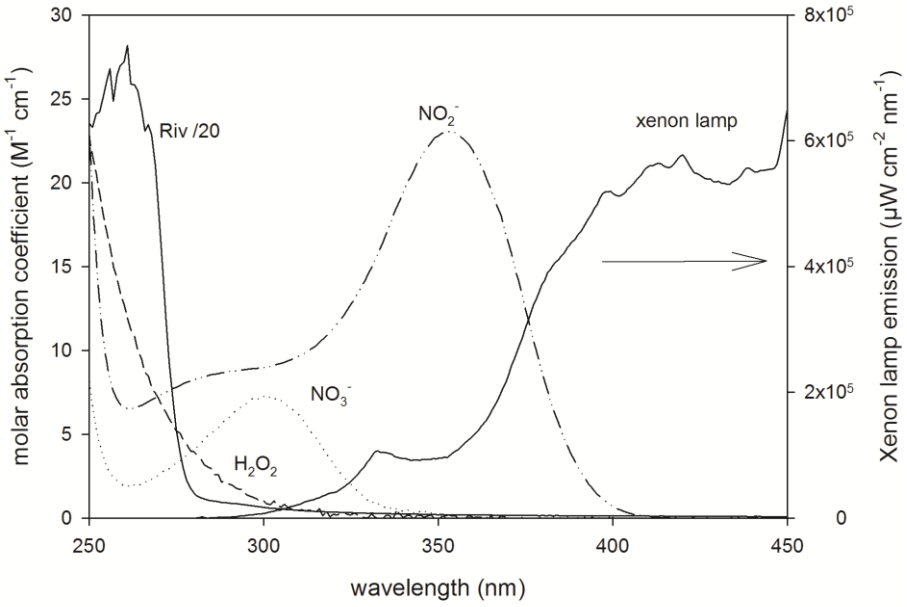
2) Initial degradation rate of Riv under polychromatic irradiation of H_2O_2 380 µM as a function of the isopropanol concentration. The solid curve shows the fit of experimental data with equation 1 and dashed lines denote the 95% confidence interval of this fit. The errors bars represent the 3σ based on the linear fit of experimental data.

3) Correlation between the initial degradation rate of Riv (R^d_{Riv}) with hydrogen peroxide and nitrite concentration under polychromatic irradiation. The errors represent the 3σ based on the linear fit of experimental data.

4) Correlation between the initial degradation rate of Riv (R^d_{Riv}) and the formation rate of hydroxyl radical ($R^f_{OH,Riv}$) reacting with rivastigmine. The photochemical sources

are: hydrogen peroxyde (empty circles) nitrites (full circles) and nitrates (empty triangles). Dashed lines denote the 95% confidence of the linear fit.

5) Proposed structures and formation mechanisms for the main rivastigmine degradation products found in the presence of hydrogen peroxide, nitrites and nitrates as photochemical sources of hydroxyl radical under polychromatic irradiation.



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462 **Figure 1**

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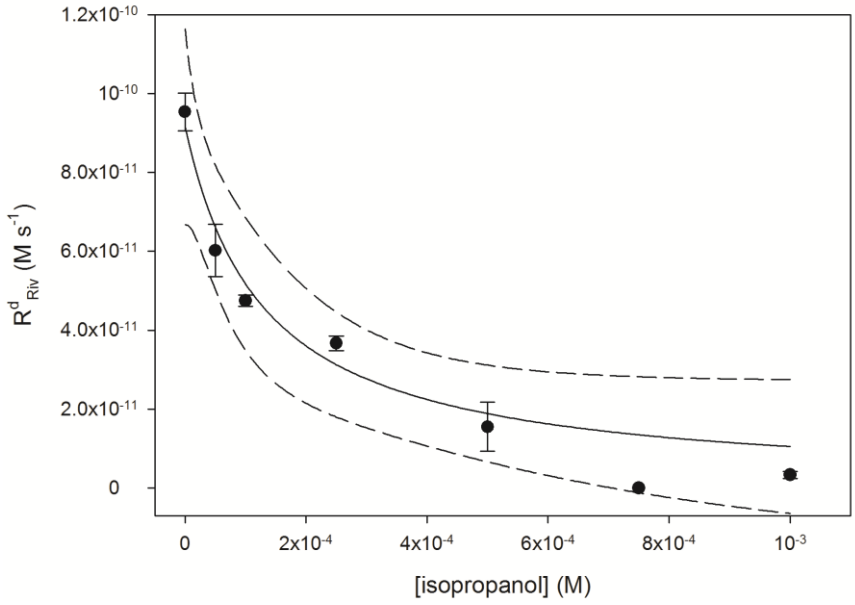

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469 **Figure 2**

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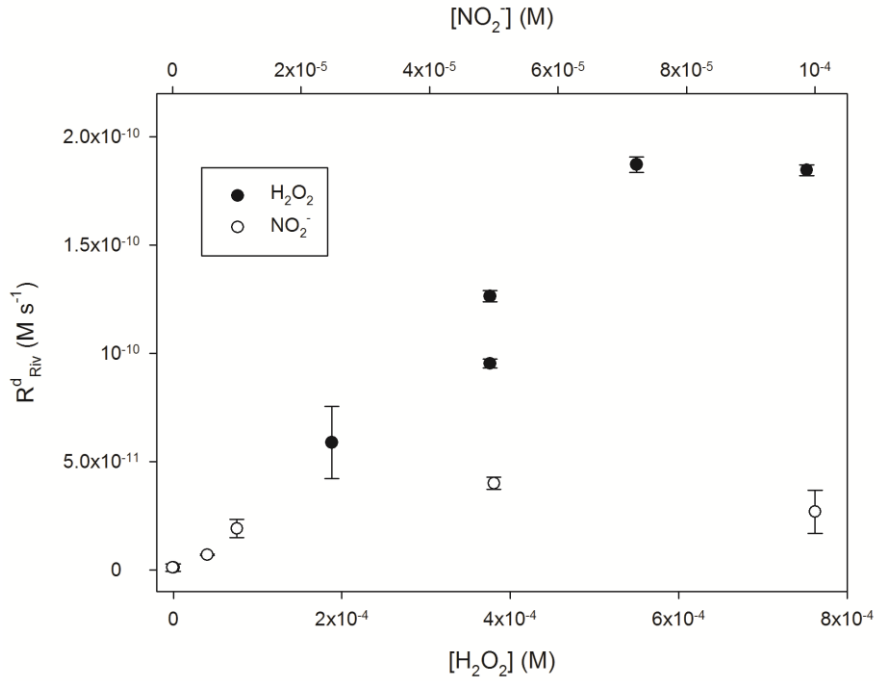
[NO2-] (M)
0
2x10-5
4x10-5
6x10-5
8x10-5
10-4
2.0x10-10
1.5x10-10
10-10
5.0x10-11
0
H2O2
NO2-
R d Riv (M s-1)
0
2x10-4
4x10-4
6x10-4
8x10-4
[H2O2] (M)

Figure 3

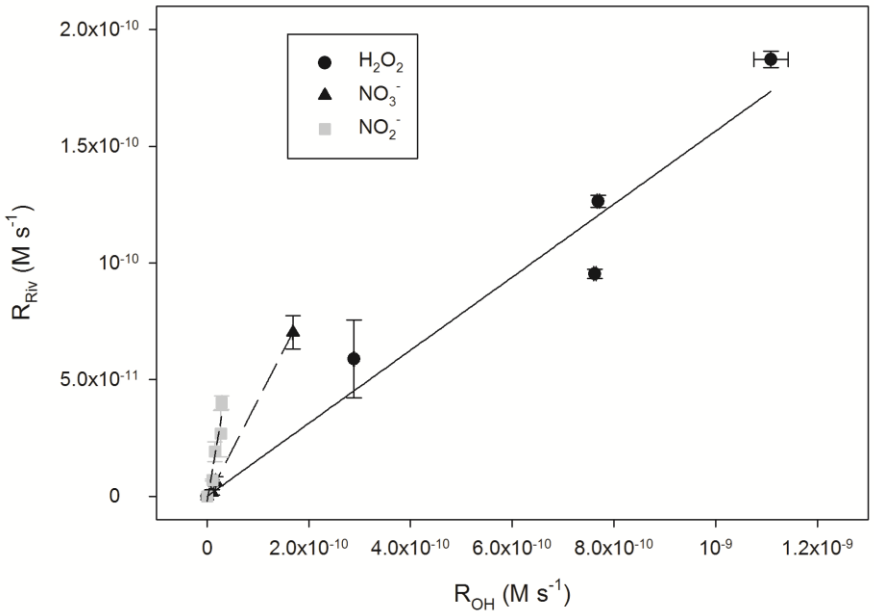
2.0x10-10
1.5x10-10
10-10
5.0x10-11
0
RRiv (M s-1)
H2O2
NO3-
NO2-
0
2.0x10-10
4.0x10-10
6.0x10-10
8.0x10-10
10-9
1.2x10-9
ROH (M s-1)

Figure 4

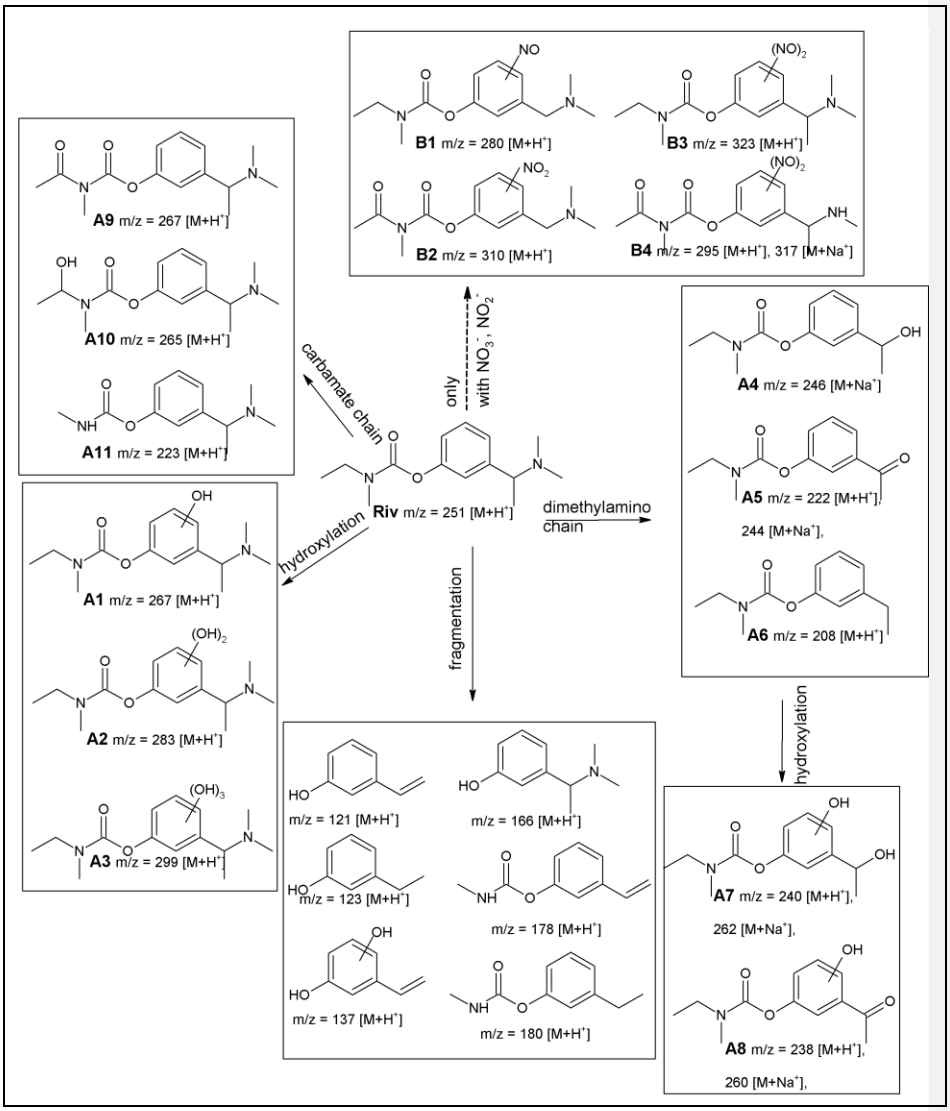
B1 m/z = 280 [M+H+]
B3 m/z = 323 [M+H+]
B2 m/z = 310 [M+H+]
B4 m/z = 295 [M+H+], 317 [M+Na+]
A9 m/z = 267 [M+H+]
A10 m/z = 265 [M+H+]
A11 m/z = 223 [M+H+]
only with NO3, NO2
carbamate chain
Riv m/z = 251 [M+H+]
dimethylamino chain
hydroxylation
fragmentation
A4 m/z = 246 [M+Na+]
A5 m/z = 222 [M+H+],
244 [M+Na+],
A6 m/z = 208 [M+H+]
A1 m/z = 267 [M+H+]
A2 m/z = 283 [M+H+]
A3 m/z = 299 [M+H+]
m/z = 121 [M+H+]
m/z = 166 [M+H+]
m/z = 123 [M+H+]
m/z = 178 [M+H+]
m/z = 137 [M+H+]
m/z = 180 [M+H+]
hydroxylation
A7 m/z = 240 [M+H+],
262 [M+Na+],
A8 m/z = 238 [M+H+],
260 [M+Na+],

Figure 5

	H_2O_2	NO_3^-	NO_2^-
k (s^{-1})	(2.35 ± 0.13) x 10^{-6}	(3.34 ± 0.21) x 10^{-8}	(2.75 ± 0.06) x 10^{-6}

Table 1: Rate constants of •OH formation from different sources (H_2O_2, NO_3^- or NO_2^-).

	Rain water	Lake water	River water
NO_3^- (M)	7.49×10^{-6}	3.53×10^{-6}	2.31×10^{-4}
NO_2^- (M)	1.15×10^{-6}	/	7.30×10^{-6}
SO_4^{2-} (M)	3.98×10^{-6}	5.09×10^{-5}	3.75×10^{-4}
Cl^- (M)	4.99×10^{-5}	1.97×10^{-4}	3.47×10^{-3}
HCO_3^-/CO_3^{2-} (M)	2.0	9.32	61.23
TOC (mg/L)	2.45	2.01	2.70
pH	7.2	6.6	7.9
$R^f_{OH\ measured}$ ($M\ s^{-1}$)[a]	$(6.42 \pm 0.53) \times 10^{-12}$	$(5.45 \pm 0.08) \times 10^{-12}$	$(3.55 \pm 0.02) \times 10^{-11}$
$R^f_{OH\ predicted}$ ($M\ s^{-1}$)[b]	3.42×10^{-12}	1.21×10^{-13}	2.80×10^{-11}
% $^{\bullet}OH$ formation from NO_3^- [c]	4.0	2.2	22.4
% $^{\bullet}OH$ formation from NO_2^- [c]	49.3	/	56.6
% $^{\bullet}OH$ formation from other [d]	46.7	97.8	21.2
R^d_{Riv} ($M\ s^{-1}$)	$(2.47 \pm 1.20) \times 10^{-12}$	$(2.83 \pm 1.63) \times 10^{-12}$	$(1.02 \pm 0.05) \times 10^{-11}$

Table 2: Concentrations of nitrite, nitrate, HCO_3^-/CO_3^{2-} and ~~Total~~ total organic carbon (TOC) of natural waters used during rivastigmine experiments. [a] $^{\bullet}OH$ formation rate obtained by using 1mM of terephthalic acid as trapping molecule. [b] Theoretical $^{\bullet}OH$ formation rate estimated by considering only NO_2^- and NO_3^- as photochemical sources. [c] Contribution to the hydroxyl radicals attributed to NO_2^- and O_3^-. [d] Contribution to the hydroxyl radicals obtained by difference from known sources.

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